ImmuKnow® as an immune monitoring tool following organ transplantation

ImmuKnow® is a non-invasive biomarker of patient cellular immune status. It helps identify transplant patients at risk of over- or under-immunosuppression after transplantation. ImmuKnow® detects intracellular ATP synthesis in stimulated CD4+ cells from whole blood. The amount of ATP produced reflects global T cell function of patients exposed to a combination of immunosuppressive drugs. The ImmuKnow® assay defines three immunological response zones: strong, moderate and weak. These zones can guide physicians in patient management. The assay can be used in a variety of applications, including transplantation, management of infectious diseases, autoimmunity, cancer, as well as vaccine and drug development.

Keywords: Cell immunity – Rejection – Infections – Solid organ transplantation.

Use of the ImmuKnow® assay has become a standard tool in transplant centers globally with utilization in 18 countries. To date, more than 120 clinical studies concerning ImmuKnow® have been performed, with over 300 publications representing more than 1,800 transplant recipients. These publications include all major allogeneic transplants (161 kidney, 66 liver, 48 heart, 27 lung, 27 pancreas, 11 small bowel/multivisceral and 8 hematopoietic stem cell) with additional abstracts in islet and composite-tissue transplant. These clinicians use ImmuKnow® to better assess changes in immune function over time and more effectively individualize immunosuppressive therapy post-transplantation. This use of ImmuKnow® results in the reduced risk of patients developing infection or rejection as well as reduction in the costs associated with these adverse events.

Over the past 30 years, improvements in immunosuppressive (ISP) therapy have helped decrease the incidence of graft rejection for organ transplant recipients, however long term graft attrition has not significantly changed during the past two decades (1, 2). Patients receiving these ISP therapies remain at substantial risk of infection, development of malignancy and serious adverse events associated with drug toxicity (e.g., kidney failure). Although therapeutic drug monitoring (TDM) is commonly used in clinical practice and trials, its primary benefit is toxicity avoidance in the absence of more effective biological markers. Additionally, routine diagnostic tests are conducted in an effort to monitor the condition of organs. Unfortunately, these markers generally lag behind the causative agent(s) of damage and indicate organ damage that has already occurred. Lastly, biopsy is invasive and the time to get conclusions is longer. Collectively, there are inherent limitations with these tools including high intra-individual variability in plasma drug levels and lack of correlation with effects on immune cell function; poor correlation between white blood cell numbers and immune function (3); and sample bias error and variability in histopathologic interpretation of biopsy specimens. While monitoring for evidence of graft dysfunction and plasma drug levels.

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of immunosuppressive drugs is useful, patients continue to present with rejection, infection and adverse reactions associated with drug toxicity. These limitations are made more complex by drugs with broad immunosuppressive effects, further compounded by utilization of multiple drug classes with unique and additive effects. The diagnostic challenge is for determining the impact of these drugs and potentially titrating them before the onset of negative biologic effects. Currently, laboratory methods exist which would allow the measurement of single or multiple markers as finely as the single cell level. Although these methods are intriguing in their potential to unlock future therapeutic and diagnostic opportunities, they far exceed the current therapeutic options and lack lab-to-lab reproducibility. The need exists for diagnostic tools which are cost-effective and matched to the range of currently available therapeutics.

A recent review by E. Wieland et al. (4) points out the importance of biomarkers which relate to the pharmacodynamic effects of immunosuppressive drugs. As stated by S. Hunt et al. (5), “an ideal immune monitoring strategy would be noninvasive, reliably allow discrimination between the presence and absence of rejection, and detect a state of over-immunosuppression.” A reliable immune monitoring assay would be a valuable tool in addition to therapeutic drug monitoring for assessment of under- or over-immunosuppression in individual post-transplant patients. Because the immune system responds quickly to change, regular and repeated patient monitoring is critical to understanding any immune response since it occurs over a period of time.

Immune monitoring for the presence or absence of rejection and detection of over-immunosuppression

The goal of immunosuppressive therapy in organ transplant is to suppress the immune response of the recipient in order to prevent graft rejection (2). Unfortunately, numerous side effects are associated with ISP therapy resulting in increased risk of infection, development of neoplasia and other comorbidities associated with decreased cellular immune function which may reduce patient survival. Therefore, it is essential to measure immune function in patients on ISP therapy in order to balance the risk of rejection with the risk of infection, malignancy and other unintended side effects related to over-immunosuppression. Although TDM is an important part of patient management, it consistently demonstrates poor utility for determining appropriate drug dose in individual patients.

Laboratory methods that are commonly employed to evaluate immune reactivity include ELISPOT assays for detection of cytokines produced by stimulated peripheral blood mononuclear cells (PBMC), lymphocyte proliferation assays (LPA), flow cytometry analysis of number and percentage of various white blood cell populations and the ImmuKnow® assay (Cylex, Inc., Columbia, MD) which measures functional capacity of cell-mediated immunity (CMI) through the activity of CD4+ cells (6-9). ImmuKnow® was developed to measure the immune reactivity of CD4+ cells in whole blood specimens using a method that is FDA-cleared, cost-effective and reproducible. ImmuKnow® measures the functional response of patient CD4+ cells to the mitogen phytohemagluttinin (PHA), and reflects an individual’s cellular immune function in the context of the biologically relevant milieu; i.e., whole blood that contains circulating levels of the immunosuppressive drugs (figure 1).

The production of intracellular adenosine-5’-triphosphate (ATP) is one of the first steps in cellular activation. Thus, the amount of ATP may be used as an early indicator of a response to immune stimuli (10). Importantly, ImmuKnow® provides a measure of immune function independent of CD4 counts, which do not always reflect the actual status of the patient’s immune system (9, 11-13).

Figure 2, p. 12 shows the odds ratios calculated for risks of infection or rejection stratified by the immune response value (ng/ml ATP) determined using ImmuKnow® for
504 recipients of solid organ transplants in a retrospective analysis performed by R. Kowalski et al. (14). The data clearly show that immune response values below 130 are associated with an increased risk of infection. Conversely, values above 450 are associated with increased risk of rejection, although with less predictive power than was observed for risk of infection as indicated by the wide confidence intervals associated with values above 450 and risk of rejection. This may partly be due to the fundamental biologic complexity of rejection and the difficulty in accurately diagnosing and histopathologically characterizing rejection. J.A. Kobashigawa et al. (11) documented differences in ImmuKnow® values in well-characterized rejections and M. Israeli et al. (12, 15) utilized a novel approach to analyze ImmuKnow® values which may help to account for some of this biologic variability (see longitudinal analysis below).

Collectively, the data indicate that serial testing with ImmuKnow® and longitudinal analyses to track changes in immune function within an individual patient provide clinically actionable information regarding under- or over-immunosuppression (16). Additionally, it may be beneficial to reduce, limit or remove organ transplant recipients from CNI-based immunosuppression, especially in those recipients with impaired renal function. Several studies demonstrate the benefit of using ImmuKnow® as an immunological marker during the conversion of patients to mammalian target of rapamycin (mTOR) inhibitors after various organ transplants (17-20).

The intent of this review is to provide practical learning regarding the most effective use of ImmuKnow®, rather than an exhaustive summary of the supporting peer-reviewed literature to date. Fortunately, liver transplantation provides a valuable scientific model for this discussion, as typically 50% of liver transplant recipients are infected with the common viral pathogen HCV.

**ImmuKnow® in liver transplantation**

As a diagnostic tool, R. Cabrera et al. reported the utility of ImmuKnow® to assist in one of the most common and challenging clinical problems in liver transplantation – discriminating between acute cellular rejection (ACR) and recurrent HCV in HCV+ liver transplant recipients (21). Blood samples were taken from 42 recipients prospectively at various times post-transplant and compared with clinical and histologic findings. In patients whose liver biopsy showed evidence of ACR, the immune response was very high; whereas, the immune response was found to be very low (p<0.0001) in those with active recurrence of HCV. The authors concluded that ImmuKnow® could potentially distinguish between ACR and HCV reactivation in those patients with abnormal transaminases and unclear histopathology.

A second question is to know whether assay results can predict which patients are at risk of HCV disease progression. A study reported by K. Hashimoto et al. described a retrospective analysis of ImmuKnow® and allograft biopsies from 54 HCV+ liver transplant recipients (22). Using a cut-off of 220 ng/ml ATP, the sensitivity and specificity for distinguishing between ACR and HCV were 88.5% and 90.9%, respectively, and the area under ROC (Receiver Operating Characteristic) curve 0.93 (CI95: 0.85-1.00). The conclusion drawn by the authors was that “the ImmuKnow® assay can be a sensitive and specific additional test for distinguishing recurrent HCV from ACR, and may be useful for predicting which recipients may be most vulnerable to recurrent HCV.”

N. Alkhouri et al. conducted a prospective study to assess the correlation between the ImmuKnow® assay and fibrosis progression in HCV+ patients post-orthotopic liver transplantation (OLT) (23). The study included 62 adult patients who underwent OLT for end stage liver disease secondary to HCV infection. **Figure 3** shows Kaplan-Meier plots illustrating the association...
between ImmuKnow® levels at 4 (A) and 12 (B) months and fibrosis progression after liver transplantation. At 12 months post-OLT, the median ImmuKnow® value was 152 ng/ml in patients with fibrosis progression compared with 264 ng/ml ATP in patients without fibrosis (p=0.008). ATP levels (ImmuKnow®) at 4 and 12 months post-OLT were significantly associated with progression of fibrosis. Lower ImmuKnow® values at 4 and 12 months post-OLT in patients with HCV recurrence were associated with more aggressive disease and rapid fibrosis progression. Assay cut-off values of 213 ng/ml ATP at 4 months and 220 ng/ml ATP at 12 months were used for their analysis. For each 25 unit increase in ATP levels at 4 and 12 months post-transplantation, the risk of fibrosis progression decreased by 22% (p=0.001), and 12% (p=0.015), respectively.

ISP therapy is the only factor associated with severe recurrence that can be modified by the clinician (24). The association of ImmuKnow® values and recurrent HCV remains statistically significant after adjustments for multiple possible confounders. These findings “may help the clinician in adjusting the immunosuppressive regimen and starting the appropriate antiviral therapy”. Collectively, the studies described by R. Cabrera, K. Hashimoto, and N. Alkhouri (21-23) show the utility of immune monitoring using ImmuKnow® for distinguishing recurrent HCV from ACR, predicting which patients may be most susceptible to liver damage associated with recurrence of HCV replication and identifying HCV+ OLT recipients at highest risk of progression of fibrosis.

The next question is: can ImmuKnow® help guide ISP changes to reduce the risk of HCV reactivation or other adverse events? Preliminary results from a prospective randomized trial in which immunosuppressive therapy (tacrolimus and steroids) post-liver transplant was modulated according to ImmuKnow® assay results versus standard of care were presented by M. Ravaïoli et al. (25). In the standard-of-care arm, clinicians were blinded to the ImmuKnow® results. The primary endpoint was the correlation among ImmuKnow® and episodes of infection or rejection and if modulation of ISP according to ImmuKnow® results was safe and effective. Patients randomized to the ImmuKnow® arm of the study had their dose of tacrolimus increased by 25% if ImmuKnow® values were >450 ng/ml ATP. ISP therapy was not changed if ImmuKnow® values were ≥130 and <450 ng/ml ATP and tacrolimus was decreased by 25% if ImmuKnow® values were ≤130 ng/ml ATP. During 2008-2011, 101 patients were enrolled into the study where 48 were randomized to the ImmuKnow® arm and 53 to the control arm. Both study groups had comparable donor and recipient characteristics including pretransplant ImmuKnow® values (91 versus 83 ng/ml ATP), age (53 versus 51 years) and MELD scores (14 versus 17). All patients enrolled had significantly lower pretransplant CMI, as measured by ImmuKnow®, than healthy controls (96±241 versus 408±184 ng/ml; p=0.02). Patients with MELD>25 had significantly lower ImmuKnow® values (median 54 versus 109 ng/ml ATP; p<0.05).

The results of the preliminary analysis are summarized in Table I. Eleven patients in the ImmuKnow® group versus 24 patients in the control group experienced infections (p<0.05). Patients who developed postoperative infections had significantly lower ImmuKnow® values at the day of transplant (median 25 versus 126 ng/ml ATP; p<0.01). Five biopsy proven rejection episodes were observed in the ImmuKnow® group versus 7 in the control group (p=NS). The results demonstrated that patients awaiting liver transplantation showed impaired cell-mediated

<table>
<thead>
<tr>
<th>Infections</th>
<th>% (p&lt;0.05)</th>
<th>Rejections</th>
<th>% (p=NS)</th>
<th>Patient survival (%)</th>
<th>Graft survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ImmuKnow®</td>
<td>11/48</td>
<td>23</td>
<td>5/48</td>
<td>10</td>
<td>87</td>
</tr>
<tr>
<td>Control</td>
<td>24/53</td>
<td>45</td>
<td>7/53</td>
<td>13</td>
<td>82</td>
</tr>
</tbody>
</table>

Table I. Incidence of infection and rejection among study groups (25).

Figure 3. Kaplan-Meier plots illustrating the association between ATP levels at (A) 4 and (B) 12 months and fibrosis progression after orthotopic liver transplantation (23).
immune response compared to healthy controls, particularly if patients had high MELD scores. Furthermore, ImmuKnow® results correlated with the infection episodes and modulation of ISP reduced the infections in the study group by almost 50% (p<0.05) with no adverse impact on acute rejection, or patient or graft survival (p=NS).

To determine whether intracellular ATP production in CD4+ cells can predict rejection or infection in transplant recipients, ImmuKnow® assay values and trough immunosuppressive drug levels were evaluated in adult liver transplant patients (N=34) during maintenance immunosuppression (26). Some of these patients (N=22) underwent gradual withdrawal from immunosuppressive treatment. Ten patients (45%) achieved complete withdrawal and the remainder (55%) achieved a 50% reduction in immunosuppressive drug use. Among the patients with episodes of rejection (N=8), none had ImmuKnow® levels in the strong immune response zone. These authors concluded by saying, “ImmuKnow® assay values are better correlated with patient response to immunosuppressive treatment than trough immunosuppressive drug levels.”

Collectively, the results derived from studies following organ transplantation suggest that appropriate testing intervals for immune monitoring using ImmuKnow® are as shown below in Table II.

**Longitudinal analysis of ImmuKnow® values**

Longitudinal monitoring with ImmuKnow® with analysis of changes in values over time is more informative than single "snap-shot" values of immune function. This was emphasized in the recent review by E. Wieland et al. (4). Numerous authors have demonstrated correlation between ImmuKnow® values and adverse events occurring within 30 days (11, 12, 14, 15, 27). S. Gupta et al. and J. Huskey et al. have attempted to correlate ImmuKnow® values with distant adverse events, and although they offer no plausible biologic mechanism for a functional marker of the immune system to have such long term predictive ability, they did demonstrate that ImmuKnow® values do not correlate with events 90 days in the future (28, 29).

M. Israeli et al. evaluated changes in ImmuKnow® values in heart transplant patients who were clinically quiescent at the time of an ImmuKnow® value and subsequently had an adverse event within 30 days (figure 4) [12]. During episodes of acute rejection, ImmuKnow® levels showed a median increase of 52% above the last ImmuKnow® measurement up to 30 days prior. This compares with infection episodes where ImmuKnow® levels showed a median decrease of 55% below the last ImmuKnow® measurement. In both scenarios, changes in ImmuKnow® were evident before any sign of rejection or infection. Most notably, CNI levels performed in parallel did not reflect the state of immunosuppression because 64% and 68%, respectively, were found within or above/below the targeted trough levels. Thus, ImmuKnow® provides an independent measurement of patient immune status.

**Cost-effectiveness of ImmuKnow®**

The cost of post-transplant patient care during the first 180 days postdischarge, including hospital readmissions if necessary, coupled with the cost of immunosuppressants and other drug therapies is estimated to total $283,100 ($77,800 and $20,600, respectively) [30]. By
using ImmuKnow®, it may be possible to significantly reduce the cost of post-transplant readmission and ISP therapy. In addition, reducing the number of tissue biopsies may also represent a substantial cost savings and benefit to the patient. In the prospective randomized trial at the University of Bologna, modulation of ISP therapy according to ImmuKnow® values reduced infection episodes by almost 50% while maintaining comparable patient and graft survival. A study performed at Porter Transplant Service in Denver (Colorado) evaluated the use of ImmuKnow® as an adjunctive diagnostic tool for identifying patients at risk for rejection or infection (31). All patients were tested preoperatively and serially after transplant. Changes in immunosuppressive therapy were made based on ImmuKnow® values, drug levels, clinical laboratory results and patient symptoms. The incidence of infection and rejection was compared to 5-year historical data. Results after 9 months of utilizing ImmuKnow® to help manage ISP demonstrated a 50% reduction in rejection and infection rates.

The group led by Anthony Sebastian, MD, at the Oklahoma University Medical Center in Oklahoma City, conducted a retrospective study to determine the impact of ImmuKnow® on reducing complications following liver transplantation (32). ImmuKnow® testing was first introduced at this institution in 2003. This group was able to study the impact of ImmuKnow® before, during and after implementation by evaluating 190 patients receiving liver transplants between 2002 and 2005 with respect to immunosuppressive drug dosing, hospital readmissions for rejection or infection, and the need for liver biopsies. Seventy-five of these patients were HCV-positive. Immunosuppressive agents were administered to achieve a target ImmuKnow® value of 280 ng/ml ATP, the point where the relative risk for rejection and infection was judged to be lowest. Prior to ImmuKnow® testing, the biopsy rate was 49 biopsies over 47 transplants (104%). Following the introduction of ImmuKnow® testing, the biopsy rate initially decreased to 25 over 59 transplants (42%), decreasing further to 18 over 63 transplants (29%) and 20 over 62 transplants (32%) following routine use of ImmuKnow®. Using ImmuKnow® also resulted in a substantial reduction in the number of hospital admissions (Table III) and length of stay, with the center benefiting from a net cost savings of $2.2 million from the use of ImmuKnow® in just the first two years ($1.1 million per year).

Table III. Nazih Zudhi hospitalization data (32).

<table>
<thead>
<tr>
<th>Liver transplant Institute</th>
<th>Number of transplants</th>
<th>Number of biopsies</th>
<th>Number of hospital days for rejections/transplant</th>
<th>Number of hospital days for infections/transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002 – ImmuKnow® Not introduced</td>
<td>47</td>
<td>49</td>
<td>2.60 (N=122)</td>
<td>1.15 (N=54)</td>
</tr>
<tr>
<td>2004 – ImmuKnow® – Standard of care</td>
<td>60</td>
<td>9</td>
<td>0.63 (N=38)</td>
<td>1.0 (N=60)</td>
</tr>
</tbody>
</table>

ImmuKnow® in kidney transplantation

Numerous investigators concluded that monitoring immune function utilizing ImmuKnow® following kidney transplantation permits therapeutic intervention which favorably affects clinical outcomes (15, 17, 33). A multicenter randomized 3-arm steroid-sparing clinical trial included 126 kidney transplant recipients (34). A multivariate analysis of the data showed that the pretransplant ImmuKnow® value was the only variable correlated with early unstable creatinine levels post-transplantation (p=0.01). Additional risk factors analyzed in this study included HLA-directed antibody testing (class I, II and DSA [Donor Specific Antibody]), number of HLA mismatches and ELISPOT responses to donor cells or normal cells. Also, recipients with ImmuKnow® values >375 ng / ml ATP were 3.67 times more likely to experience an ACR (p=0.03). Based on these results, it appears that pretransplant assessment of donor-specific and nonspecific immune parameters may identify which kidney recipients may benefit from closer clinical and immunological scrutiny and more careful tailoring of immunosuppressive therapy to optimize short- and long-term graft outcome. In renal transplantation, many centers test patients at regular intervals for BK viruria and/or viremia by PCR. Once BK is detected in the blood, immunosuppression is empirically reduced by decreasing or discontinuing one or more agents. Using ImmuKnow®, a preliminary study stratified kidney transplant recipients accur-
ImmuKnow® in heart transplantation

J.A. Kobashigawa conducted a single center prospective observational analysis of 296 heart transplant recipients with 864 ImmuKnow® values and demonstrated the mean ImmuKnow® value was significantly lower during infection (N=38; 187 versus 280 ng/ml ATP; p<0.001) than steady-state and somewhat higher during rejection (N=8; 327 versus 280 ng/ml ATP; p=0.35). ROC curve analysis calculated a sensitivity of 71% and specificity of 73% for ImmuKnow® values <200 ng/ml ATP for diagnosis of infection. Area under ROC curve was 0.73. While there were too few rejection events for statistical analysis, 3 of the 8 episodes of rejection were antibody-mediated with hemodynamic compromise. Additionally, the mean ImmuKnow® value was significantly higher in these patients than in steady-state patients (491 versus 280 ng/ml ATP; p<0.001). These investigators concluded that ImmuKnow® predicts the risk of infection in heart transplant patients.

A prospective study of 50 heart transplant recipients found sequential ImmuKnow® values provided more useful information than single “snap-shot” values. ImmuKnow® was used as a clinical biomarker that was incorporated into routine follow-up examinations and anytime a patient’s clinical status was considered unstable or required modifications in immunosuppressive therapy. As seen in other cross-sectional studies, ImmuKnow® values obtained during periods of clinical stability (351 ng/ml ATP) were significantly different than values obtained during episodes of rejection (619 ng/ml ATP) or infection (129 ng/ml ATP; p<0.05). Furthermore, changes in sequential ImmuKnow® values with a median decrease or increase of 55% were prognostic of infection or rejection respectively. Each of these changes in ImmuKnow® values preceded all other routine clinical assessments.

An additional prospective study in heart transplant recipients used routine testing with ImmuKnow® to guide conversion from CNI to everolimus (12). Heart recipients (N=36) were converted to everolimus due to worsening pients. Under standard triple therapy of tacrolimus, MMF and steroids following induction therapy with basiliximab, patients with low ImmuKnow® values had significantly more infections, including CMV disease, than patients with higher baseline ImmuKnow® values. At one year, reduced ImmuKnow® values predicted increased risk of infection, particularly CMV replication; the data showed correlation between stable ImmuKnow® values and lower serum creatinine levels.
renal function, cardiac allograft vasculopathy, recurrent CMV infections, malignancy or CNI-induced neuropathy. Average ImmuKnow® values during CNI therapy (maintenance), preconversion and 3 months postconversion to everolimus were 329±148 (range 48-600), 365±181 (range 33-940) and 355±120 (range 44-888) ng/ml ATP respectively (p=NS). During CNI therapy 13 infections and 5 rejections occurred within the 3 months prior to initiation of the conversion protocol. Postconversion, there were 2 infections and 1 rejection (2.1% and 0.8% versus 1.8% and 0.9% per patient month, p=NS).

**ImmuKnow® in lung transplantation**

A prospective observational study assessed the sequential use of ImmuKnow® in stable and infected lung transplant recipients (41). Samples (N=143) were obtained from 57 clinically stable patients up to 703 (±695) days following lung transplantation. The average ImmuKnow® value was 244±138 ng/ml ATP. Of these patients, 15 developed various infections or co-infections including bacterial pneumonia (N=10), fungal pneumonias (N=3) and CMV infections (N=2). At the time of their infection, these patients had significantly lower ImmuKnow® values compared with the non-infected patients (111±83 ng/ml ATP versus 283±143 ng/ml ATP; p<0.0001). By reducing tacrolimus doses from an average of 7.2±5.1 mg/day to 5.2±4.1 mg/day with concomitant decrease of trough values from 6.9±3.1 ng/ml to 4.3±1.9 ng/ml, ImmuKnow® values increased on average from 173±97 to 215±100 ng/ml ATP. In all cases, the infections resolved and no rejections occurred. Additionally, no correlation between the tacrolimus dose or trough levels and ImmuKnow® values was noted. Eleven patients who developed infections had sequential ImmuKnow® values. Upon resolution of the infection, the ImmuKnow® value increased consistent with an improved immune response against infection.

To test the hypothesis that global immunity varies among patients with different infectious syndromes, a large cohort of 175 lung transplant recipients was prospectively monitored with ImmuKnow® (27). ImmuKnow® values (N=710) were obtained over a period of 2 to 48 months post-transplant. All patients received alemtuzumab induction followed by tacrolimus and prednisone maintenance ISP therapy. Valgancyclovir and voriconazole were used for CMV and fungal prophylaxis, and trimethoprim/sulfamethoxazole for *Pneumocystis pneumonia* prophylaxis. Only ImmuKnow® values obtained within a month of an infectious episode were used in the analysis. During the course of the study, 129 infections occurred in 73 lung recipients. The median ImmuKnow® values in these patient groups are summarized in **Table IV**. The most significant differences occurred among patients with CMV pneumonia. Multivariate risk hazard analysis showed the risk of infection was increased in patients with ImmuKnow® values <100 ng/ml ATP. Of note, patients with fungal colonization had similar ImmuKnow® values to stable patients; however, those who subsequently developed fungal disease within 100 days had significantly lower values (22.5 versus 183.5 ng/ml ATP; p<0.0001). It is very difficult to determine if lung transplant recipients simply have fungal colonization or if the fungus is leading to or causing disease. In the absence of disease, lung transplant recipients with detectable fungus and with ImmuKnow® values comparable to stable patients did not develop disease; whereas, in those patients in which fungus was detected and ImmuKnow® values were low, progression to fungal disease occurred over the next 100 days.

**Conclusion**

Together, the information presented here demonstrates that a significant relationship exists between ImmuKnow® assay values and global immune function following solid organ transplantation. ImmuKnow® is a standardized, FDA-cleared, noninvasive assay that allows immune management of therapy according to the individual patient’s immune status (12, 42). Proactive therapy management coupled with a serial decrease in ImmuKnow® values enables the clinician to consider modifying ISP to avoid the infectious events (43, 44). Furthermore, studies have demonstrated, in the setting of OLT, the utility of ImmuKnow® for distinguishing recurrent HCV infection from other viral infections, and identifying HCV+ OLT recipients at highest risk of fibrosis progression. Lastly, preliminary results from a prospective, randomized, controlled study suggested that Proactive therapy management coupled with serial decrease in ImmuKnow® values enables the clinician to consider modifying ISP to avoid the infectious episodes associated with recurrence of HCV infection from other viral infections, and identifying HCV+ OLT recipients at highest risk of fibrosis progression.

### Table IV. ImmuKnow® values in lung transplant recipients with viral, fungal or bacterial infections (27).

<table>
<thead>
<tr>
<th>Cohort</th>
<th>N</th>
<th>ImmuKnow®, ng/ml ATP (median, 25th-75th percentile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable patients</td>
<td>581</td>
<td>174.8 (97-306)</td>
</tr>
<tr>
<td>Cytomegalovirus, other viruses</td>
<td>13</td>
<td>49.3 (4-120)</td>
</tr>
<tr>
<td>Aspergillus, other fungal infections</td>
<td>61</td>
<td>167.6 (88-251)</td>
</tr>
<tr>
<td><em>P. aeruginosa</em>, other bacterial infections</td>
<td>14</td>
<td>92.4 (55-130)</td>
</tr>
</tbody>
</table>
interventional trial indicate that ImmuKnow® may be helpful in decision-making related to modulation of immunosuppressive therapy in order to reduce adverse effects of over-immunosuppression and improve clinical outcomes. ImmuKnow® is a cost-effective diagnostic tool, well-matched to the currently available range of therapeutics. Through proper use of ImmuKnow®, clinicians have another tool they can use to impact patient care starting with the way they optimize treatment decisions for their transplant patients. Improved patient management makes it possible to reduce the cost of post-transplant admission and immunosuppressive therapy, the added expense associated with infection-related hospitalizations (45) and to avoid the potential complications associated with organ biopsy (46).

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